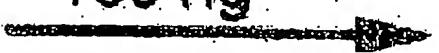
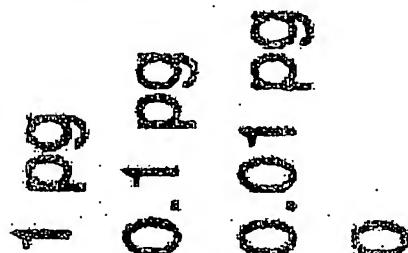


FIG. 1. Sensitivity of the PCR assay. Shown are the results of PCR amplification of the serially diluted *Z. m. mawisi* (DD8) DNA, and on negative gels. DNA was extracted from parvovirus cultures and purified as described in Materials and Methods. Lane M, 1 kb ladder (Gibco BRL); Lane 1, 10  $\mu$ g of DNA; Lane 2, 1  $\mu$ g of DNA; lanes 3, 10 ng of DNA; lane 4, 1 ng of DNA; lane 5, 10 pg of DNA; lane 6, 1 pg of DNA.

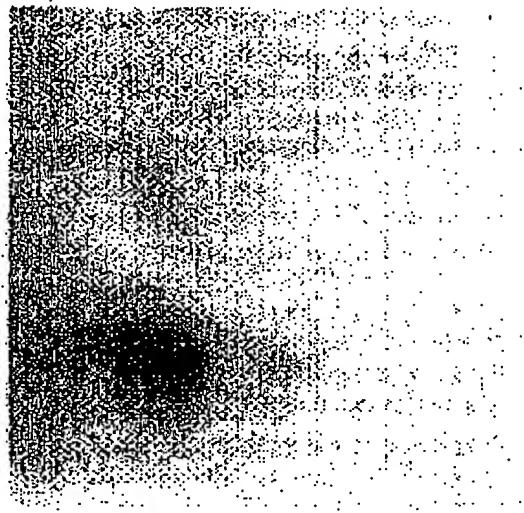
Probe: Ld Ind kDNA

Human DNA: 100 ng 

Primer Set: LdI1 & 2

Amt. Ld Ind DNA:   
1 pg 1 pg 1 pg  
10 pg 10 pg 10 pg  
100 pg 100 pg 100 pg

(Kb)  
0.87 —  
0.6 —



1 2 3 4

FIG. 2. Sensitivity of PCR amplification of *Leishmania* kDNA followed by Southern blot analysis. The PCR contained 100 ng of human genomic DNA and the indicated amount of total DNA from *L. donovani* DD8. The PCR product was probed with parasite kDNA and exposed for about 1 h. Lane 4 represents a PCR containing only human DNA as a control.

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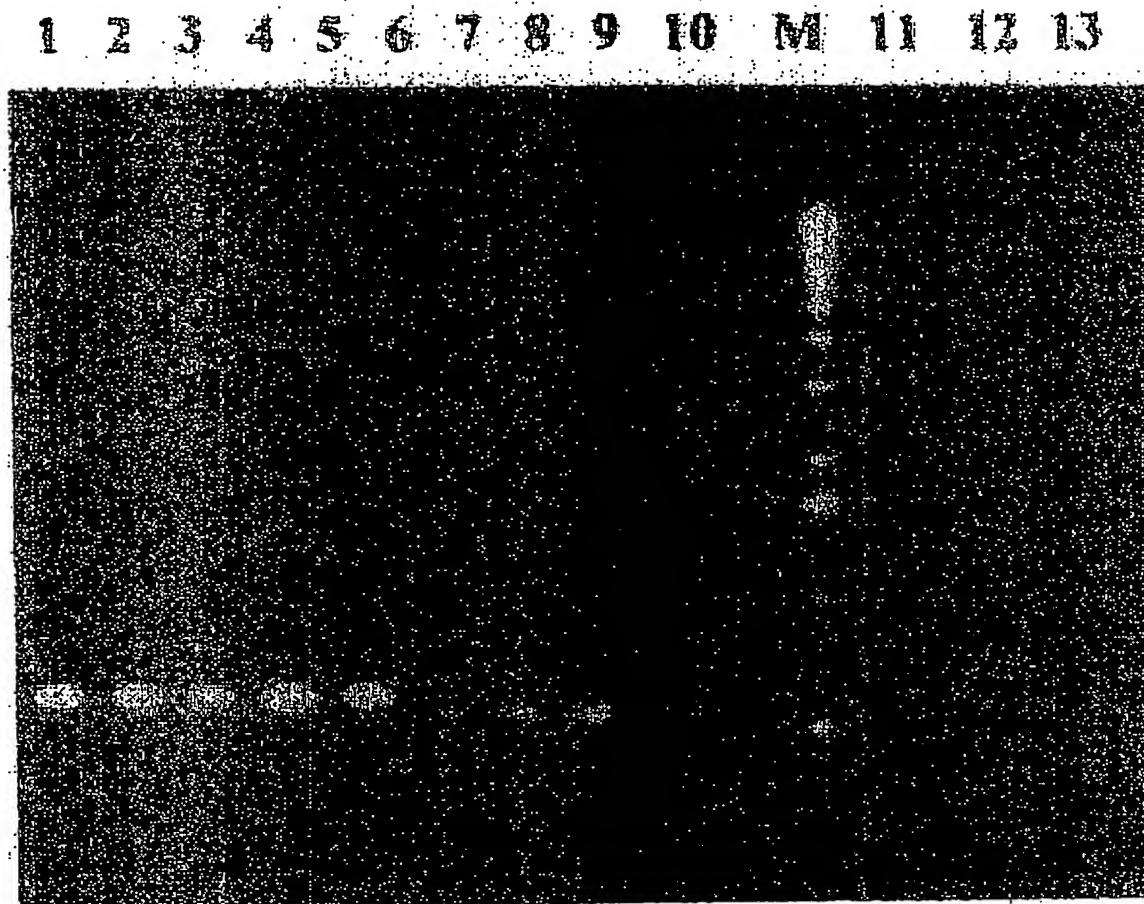


FIG. 3. Amplification of parasite DNA from various strains and isolates of *Leishmania*. DNA (1 ng) isolated from parasite cultures was injected to PCR and analyzed. Lane 1, *L. donovani* AG83; lane 2, *L. donovani* DD8; lane 3, *L. donovani* IICB8; lane 4, *L. donovani* CB6; lane 5, *L. donovani* ICB 7 (PKDL origin); lane 6, *L. donovani* S; lane 7, *L. donovani* WR684; lane 8 *L. donovani infantum*; lane 9, *L. tropica* WR683; lane 10, *L. major* L.V. 39; lane M, 1-kb ladder; lane 11, *Plasmodium*; lane 12, *M. leprae*; lane 13, *M. tuberculosis*.

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M 1 2 3 4 5 6 7 8 9 10 11

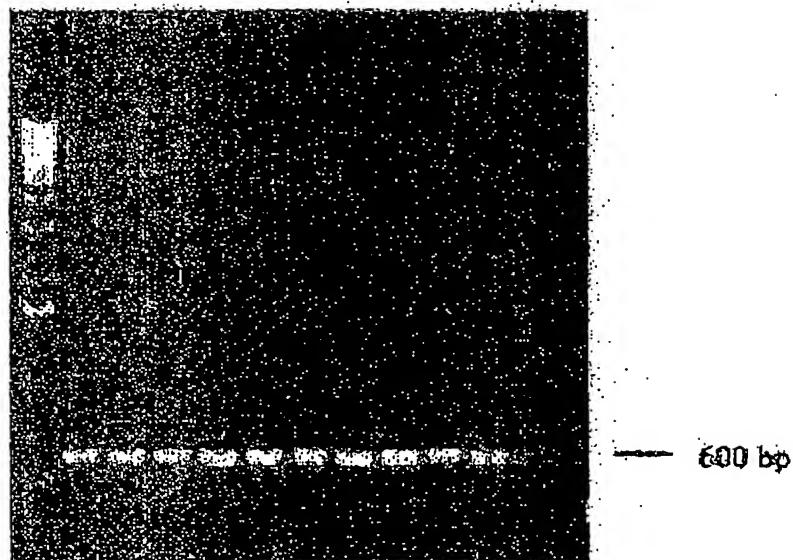


FIG. 4. DNA amplification from recent field isolates of KA and KDL. DNA (1 ng) extracted from cultures of parasite isolates was used for PCR amplification. Lanes: M, 1-kb ladder; 1, KA-1; 2, KA-2; 3, KA-3; 4, KA-4; 5, KA-5; 6, PK-1; 7, PK-2; 8, PK-3; 9, PK-4; 10, PK-5; 11, isolate from a patient with cutaneous leishmaniasis.

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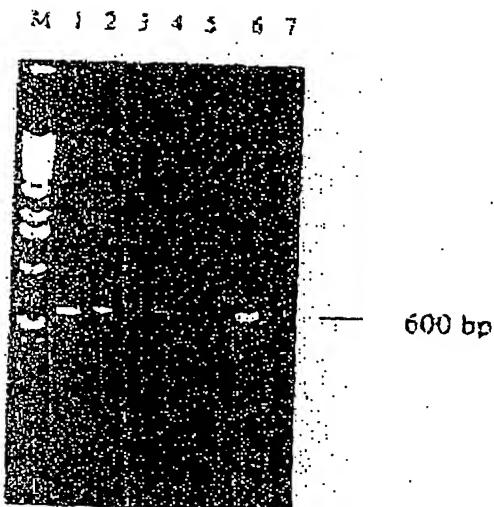


FIG. 5. PCR assay with clinical samples of KA and PKDL DNA (100 ng) isolated from clinical samples was used for PCR amplification. Lane M, 1-kb ladder; lane 1, KA (bone marrow); lane 2, KA (blood); lane 3, malaria (blood); lane 4, tuberculosis (blood); lane 5, control from the area of endemicity (blood); lane 6, PKDL (skin lesion); lane 7, leprosy (lesion).

45000-35000-25000-15000-5000

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Fig. 6. Sequence of PCR products with DNA isolated from *L. donovani* DD8 strain, isolates and clinical samples of KA and PKDL.

1 gaatttcggcg aaaaatgacc gaaaatgggc caaaaaccca aacttttctg gtcccttcgggg  
61 tagggggcggtt ctgcgaaaaac cgaaaaatgg gtgcagaaat cccgttcaaa aaatagccaa  
121 aaatgcacaa atcggttcc gaggcgggaa aactgggggtt ggtgtaaaat agggtcgggt  
181 ggaggggaaa ttccggggccc ggacgtgtgt ggatatggcc tgggtggggaa ctttggatcg  
241 ggttgtactt gtatgggggtt tggacctgg ctgggggttt ggggggttgggt gtggggaaagg  
301 ggttgtgggtt atttggagtg acgttggctt ttttgcataat tgatatttgc ttbaaactgg  
361 atttggctcggt ctggatcacac gttgggttggg ttggatctgg attggatcttg gatcttgcac  
421 ggggtccggag gcttgatctg ggggtggagga gtttgggggg aragttttgg atgtttagtac  
481 ggaatgttgc ttcttttaat ataaatatta gttggggcgtt ttgcatttgc ttgtttccacg  
541 ggagtagcct caggacttta ggcgggagat actatattat cggtagtata atatccataag  
601 tatacgttar agatataatgt taattgttagt atattgtaga ttatgttac agtgtatag  
661 ctatgtttt actatgtata atttgtattt gatgtatag tgctactgt agagtgtacc  
721 tttcaatgtt atatgtatgt ctgtttttcc tttatgggt gggaaatgggt gtgaggggctg  
781 gaaatgttcc ttgttcc